Localization of talin in skeletal and cardiac muscles

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Antibodies to talin and vinculin were used for localization of these proteins in skeletal and cardiac muscles by the indirect immunofluorescence method. We have found that talin is localized in intercalated discs of cardiac muscle and in costameres of skeletal and cardiac muscles. It is suggested that in striated muscles talin and vinculin play an important role in interactions between actin filaments and membranes.

(Cardiac muscle, Skeletal muscle) Talin Vinculin Immunofluorescence Microfilament Membrane-protein interaction

1. INTRODUCTION

Studies on membrane-microfilament interactions have focused a lot of interest on the protein vinculin. Vinculin was localized to the termini of actin filaments in focal contacts of cultured cells, zonula adherens of intestinal epithelium, intercalated discs of cardiac muscle and dense plaques of chicken gizzard smooth muscle [1–5]. Vinculin has also been identified in specific regions of attachment between striated muscle myofibrils and the surrounding membrane of sarcolemma [6–8]. In skeletal and cardiac muscles vinculin was found in a two-dimensional orthogonal membrane lattice – the transverse elements of the vinculin lattice being called costameres [6–8]. The membrane-associated costameres are highly organized and periodic with the underlying sarcomeres which overlie the I band of the sarcomere [6–8]. The costameres appear to be physically coupled to the actin filaments of sarcomeres [6–8]. Recently a new protein in adhesion plaques of cultured cells was discovered which was referred to as talin [9]. Talin was found to have an M_r of 215000 [9]. Talin forms a complex with vinculin with a dissociation constant of about 10^{-8} M [10]. Talin, however, does not bind to actin [9,10].

Here we present the results of immunofluorescence localization of talin in avian skeletal and cardiac muscles. We report that talin is localized in intercalated discs of cardiac muscle and in costameres of skeletal and cardiac muscles.

2. MATERIALS AND METHODS

The preparation and characterization of rabbit affinity-purified antibody to chicken gizzard vinculin have been described [7]. The rabbit antiserum to chicken gizzard talin used in this study was a gift from Dr K. Burridge (University of North Carolina, USA). The preparation and characterization of antitalin antiserum have been described [9]. The affinity-purified antibody to vinculin and antiserum to talin were characterized by standard immunological techniques and in all tests interacted only with vinculin (antivinculin antibody) or with talin (antitalin antiserum) [7,9]. The antitalin antiserum and affinity-purified antibody against vinculin were found to interact only with talin and vinculin, respectively, in immunoblots of SDS-soluble proteins from chicken cardiac and skeletal muscles (not shown).

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For immunofluorescence staining 3–4 μm longitudinal and transverse sections from frozen chicken cardiac and skeletal muscles were cut on a cryostat at −20°C and mounted on glass slides. After incubation with 0.04 mg/ml affinity-purified antivinculin IgG or antitalin antiserum (dilution 1:400) for 60 min, sections were washed and then fluorescein-conjugated goat anti-rabbit IgG added. After incubation for 45 min sections were washed and viewed in a Zeiss epifluorescence photomicroscope III with 40× and 60× objectives. To intensify the fluorescence, sections were incubated for 45 min with fluorescein-conjugated rabbit anti-goat IgG.

3. RESULTS AND DISCUSSION

To localize talin in striated muscles, cryostat sections of chicken skeletal and cardiac muscles were studied by indirect immunofluorescence using antiserum to chicken smooth muscle talin. When transverse sections were examined (fig.1A,C) we observed that fluorescence was confined to the cell margins. No staining is observed intracellularly in the fibers. Occasional bright patches of stain (cardiac muscle, fig.1A) correspond to vessels. The localization pattern of talin is similar to that of vinculin (fig.1B,D). Staining for talin was significantly less intense compared to vinculin. In

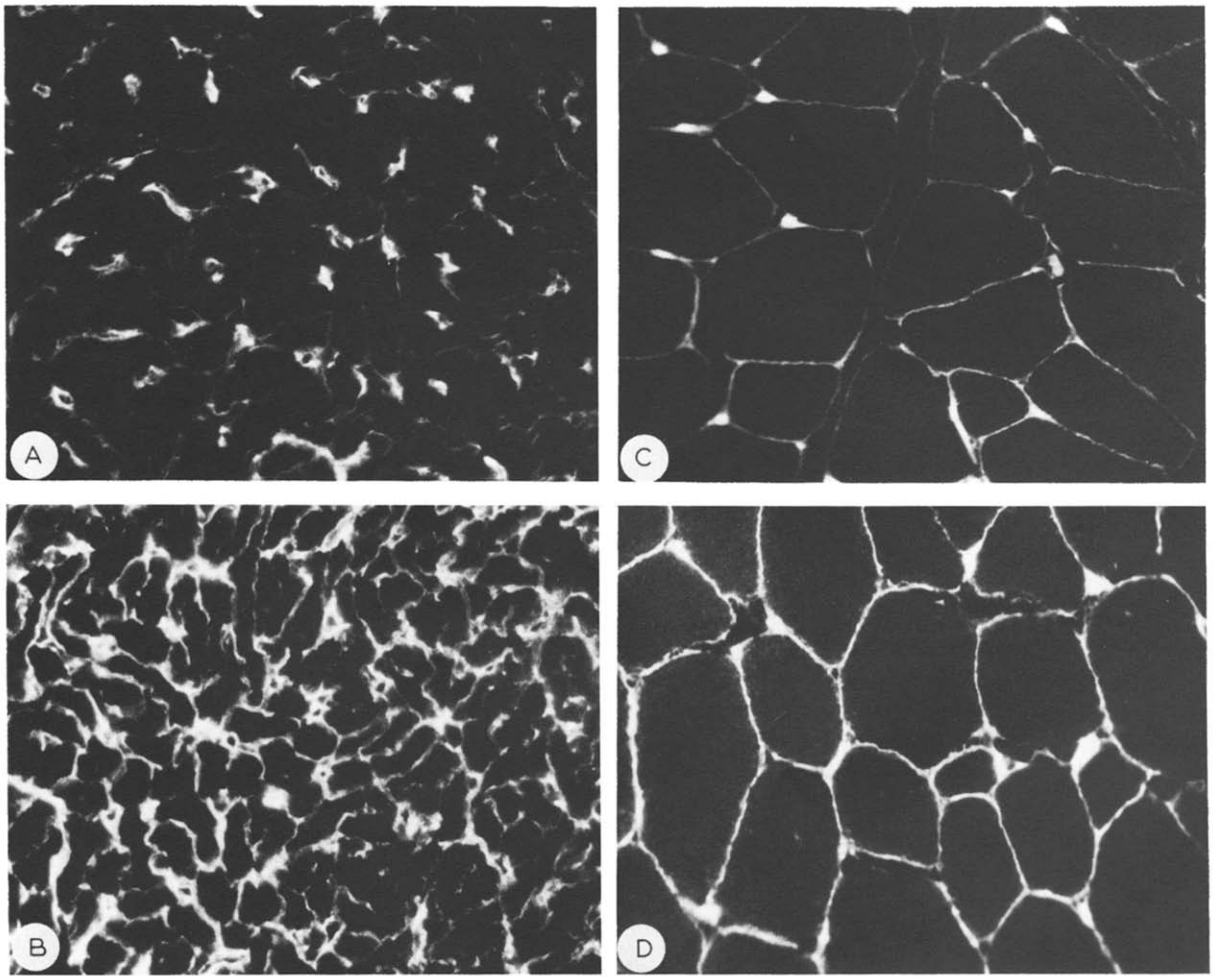

Fig.1. Immunofluorescence localization of talin and vinculin in chicken striated muscles. Transverse section of: (A) cardiac muscle stained for talin, × 810; (B) cardiac muscle stained for vinculin, × 810; (C) skeletal muscle stained for talin, × 910; (D) skeletal muscle stained for vinculin, × 910.

transverse sections of striated muscles vinculin is detectable only at the sarcolemma (fig.1B,D) [6–8]. Because there was no difference in the distribution of talin and vinculin in serial transverse sections of striated muscles we conclude that talin is also concentrated very close to the sarcolemmal membrane. Immunofluorescence analysis of longitudinal sections of chicken skeletal and cardiac muscles demonstrates different types of talin localization in striated muscles. Talin is located in specific cardiomyocyte contact regions – in intercalated discs (figs 2B,3C). The periphery of the myocytes along the cell margins was stained by the antibody to talin (fig.2A). The membrane-associated talin in a two-dimensional lattice with longitudinal and transverse periodicity was found in sections that provide a surface view of the cell (figs 2A,B,3D). The longitudinal sections selected to contain the surface of the fibers demonstrate that antitalin staining near the sarcolemmal membrane is periodic and corresponds to the I bands of sarcomeres around the Z-line region (fig.3A,C) [the phase-contrast micrograph (fig.3B) illustrates that fluorescent bands in fig.3A overlie I bands of subjacent myofibrils]. Talin was not found on internal or glycerinated myofibrils (figs 2,3E,F). It should be mentioned that in parallel to antitalin staining of striated muscle sections the same serial longitudinal sections were stained with antivinculin. For both vinculin and talin the immunofluorescence staining was very similar (not shown), the only difference was the intensity of the staining. Staining for vinculin in muscles was obviously greater compared to talin. Based on these co-localization studies, we conclude that in striated muscles talin could be a structural component of myofibril-membrane attachment sites defined earlier by the localization of vinculin [4–8]. One of those sites is intercalated discs in cardiac muscle, another being the transverse components of the lattice of myofibril-to-sarcolemmal membrane attachment sites (costameres) in skeletal and cardiac muscles. Recently a number of new proteins were identified in intercalated discs of cardiac muscle and in costameres of striated muscle, including filamin, α-actinin, desmin, vinculin, 200 kDa protein – for intercalated discs and vinculin, γ-actin, spectrin, intermediate filament antigens – for costameres [3,5–8,11–13]. It was shown that in intercalated discs vinculin is more closely associated with the membrane than other proteins and is located at the fascia adherens of the intercalated disc membrane [3,5]. On the other hand, vinculin and talin are two proteins that interact with each other [10]. Based on these data and results obtained here we suggest that in striated muscles a complex of vinculin and talin may form a peripheral domain playing an important role in the

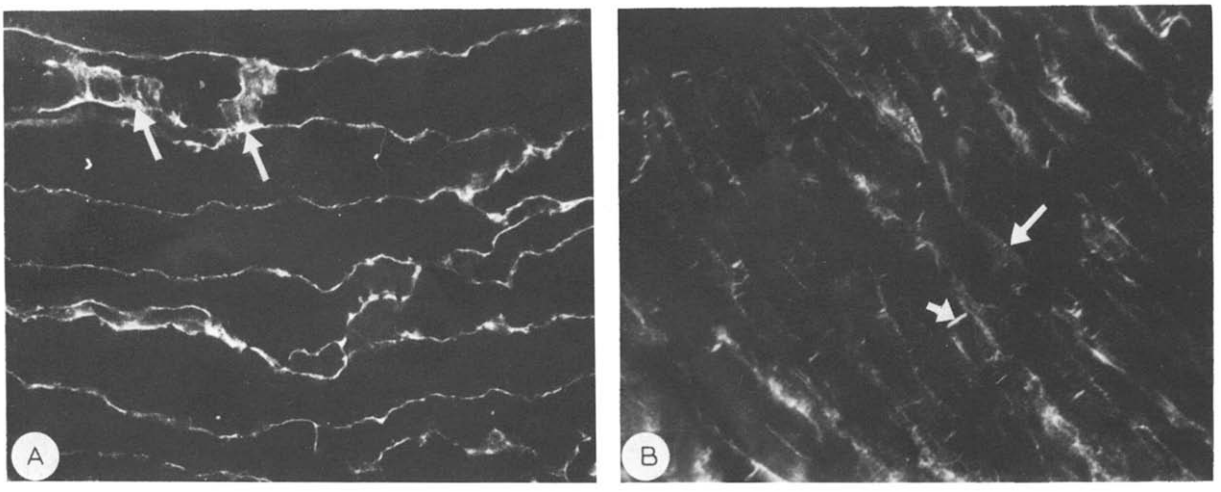

Fig.2. Immunofluorescence localization of talin in chicken skeletal (A) and cardiac (B) muscles (longitudinal sections). In (A) talin is associated with cell margins; arrows, presence of talin in sarcolemma; × 770. In (B) talin is associated with intercalated discs (small arrow) and with costameres (large arrow), × 1100.

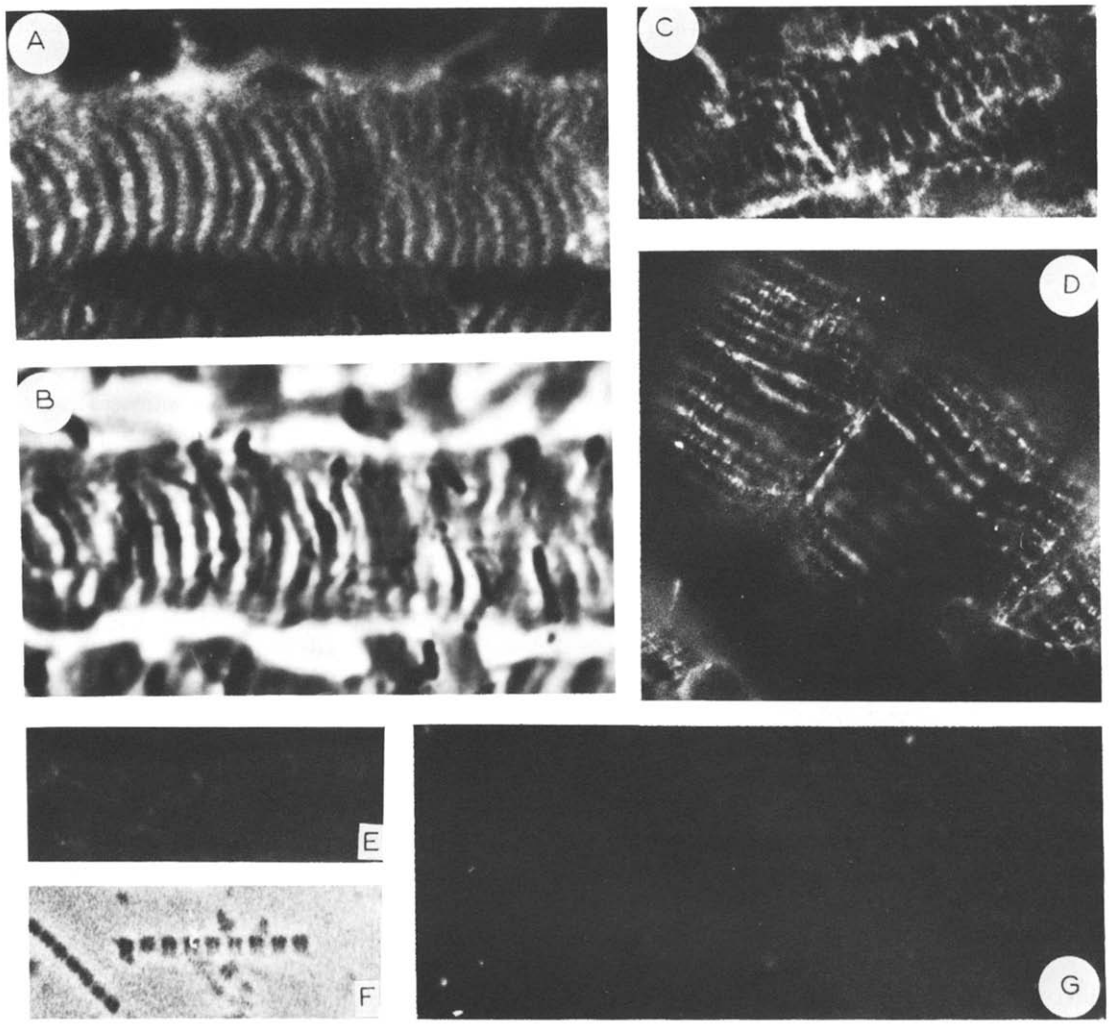

Fig.3. Costameres in chicken skeletal and cardiac muscles (longitudinal sections). (A) Skeletal muscle, the costameres are periodic and overlie the I band, immunofluorescence. (B) Phase-contrast micrograph of (A), × 2200. (C) Cardiac muscle discontinuous costameres, × 3350. (D) Skeletal muscle; talin is organized at the sarcolemma in a two-dimensional lattice, × 2800. (E,F) Glycerinated chicken skeletal muscle myofibrils, (E) immunofluorescence, (F) phase contrast, × 390. (G) Chicken skeletal muscle incubated with non-immune IgG, × 2200. No staining was observed when antitalin antiserum was pre-adsorbed with talin.

attachment of microfilaments to membrane in two specialized structures of skeletal and cardiac muscles.

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